Published in final edited form as:

Chembiochem. 2016 March 15; 17(6): 462–465. doi:10.1002/cbic.201500656.

Juxta-Terminal Helix Unwinding as a Stabilizing Factor to Modulate the Dynamics of Transmembrane Helices

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Abstract

Transmembrane helices of integral membrane proteins often are flanked by interfacial aromatic residues that may serve as anchors to aid the stabilization of a tilted transmembrane orientation. Yet physical factors that govern the orientations or the dynamic averaging of individual transmembrane helices are not well understood and have not been adequately explained. When using solid-state ²H NMR spectroscopy to examine lipid bilayer-incorporated model peptides of the GWALP23 (acetyl-GGALW(LA)₆LWLAGA-amide) family, we observe substantial unwinding at the terminals of several tilted helices spanning the membranes of DLPC, DMPC or DOPC lipid bilayers. The fraying of helix ends may be vital for defining the dynamics and orientations of transmembrane helices in lipid-bilayer membranes.

Graphical Abstract

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The authors declare no conflict of interest.

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Helix end fraying is significant for stabilizing the orientations and limiting the dynamics of transmembrane helices in membrane proteins.

Keywords

solid-state deuterium NMR; tryptophan; transmembrane helix unwinding; GWALP23 peptide; protein-lipid interaction

It has been noted that the dynamic properties of closely similar transmembrane helices may differ widely ^[1–4]. Although the mechanisms that govern the orientations or the extent of helix dynamic averaging have not been well understood, dynamic and structural features are both important for defining the functional properties of membrane proteins. While the detailed issues are puzzling, there have been some indications that having “too many” interfacial Trp or Tyr residues may correlate with high levels of dynamic averaging ^[3–5].

Meanwhile, it has been recognized that the aromatic Trp and Tyr residues of membrane proteins favor the membrane/water interface ^[6–9], although the energetic stabilization provided by the interfacial interaction is likely to be modest. Namely, when the partitioning energies for the imidazole or phenol side chains between water and either cyclohexane ^[10–11], the membrane interior ^[12] or octanol ^[13–14], representing an interfacial polarity ^[15], are compared; the observed differences fall in a range of only about 0.6–1.8 kcal/mol. It was therefore initially somewhat surprising to note a well-defined, tilted transmembrane orientation for the helix of acetyl-GGALW(LA)₆LWLAGA-amide (“GWALP23”) in several lipid-bilayer membranes ^[16–17]. Furthermore, it is remarkable that GWALP23 exhibits substantially less motional averaging of solid-state NMR observables than do similar 23-residue helices that have four Trp residues instead of two ^[3–4]. The results observed to date raise a lingering question: What factors may stabilize a tilted transmembrane orientation for neutral peptides such as GWALP23 in lipid bilayers? A further puzzle is presented by the well-defined orientations of the single-Trp peptides acetyl-

GGALF(LA)₆LWLAGA-amide and acetyl-GGAFF(LA)₆LWLAGA-amide (“F⁵GWALP23” and “F^{4,5}GWALP23”) in lipid bilayer membranes [5], whereas, by contrast, the corresponding Y^{4,5}GWALP23 helix exhibits excessive dynamic averaging which in turn precludes a well-defined average orientation [18].

We sought to address additional factors, beyond the role of the interfacial Trp or Tyr aromatic rings (as opposed to Phe), for stabilizing the preferred orientations and limiting the dynamics of single transmembrane helices. To this end, we first prepared acetyl-GGAAA(LA)₆LWLAGA-amide (A^{4,5}GWALP23;) with specifically deuterated Ala residues within the core (LA)₆L helical region. We examined whether A^{4,5}GWALP23 would have a distinctly favored orientation in bilayer membranes and whether the extent of dynamic averaging, in particular rotational diffusion about the helix axis [3–4], would be large or small. Importantly, A^{4,5}GWALP23 contains no charges and only one aromatic residue (W19). Later we incorporated deuterium labels at A3 and A21 of A^{4,5}GWALP23 as well as F^{4,5}GWALP23, as a control, for the purpose of examining whether the peptide terminal segments are helical or unwound (“frayed”) when in the bilayer membrane environment, while noting that Phe has a much weaker preference than Trp or Tyr for the interface [5, 8, 19–20].

The designed peptides were successfully synthesized and purified, using methods that have been described [5]. Peptide purity and identity were confirmed by liquid chromatography and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (Figures S1 and S2 of the Supporting Information). The ³¹P NMR spectra from macroscopically aligned lipid-peptide samples (Figure S3 of the Supporting Information) confirm that the lipid head groups are organized within well-oriented lipid bilayers. Circular dichroism spectra (Figure S4 of the Supporting Information) indicate that bilayer-incorporated A^{4,5}GWALP23 is largely helical, which then allows characterization of the behavior of the peptide helices in aligned bilayers by means of solid-state ²H NMR spectroscopy. The ²H quadrupolar splitting magnitudes $| \nu_q |$ from the labeled alanine CD₃ groups serve to define a preferred tilted, dynamically averaged, orientation of the core helix with respect to the bilayer normal in an applied magnetic field [17].

From the ²H NMR spectra of oriented liquid-crystalline peptide-lipid samples (Figure 1), it is evident that the single-Trp peptide A^{4,5}GWALP23 as well as F^{4,5}GWALP23 (observed previously) [5] aligns well in the lipid bilayers and undergoes the typical rapid reorientation about the bilayer normal [21–22], yet experiences surprisingly little additional dynamic averaging. Extensive dynamic averaging would narrow the range of $| \nu_q |$ values [21], but the $| \nu_q |$ range for A^{4,5}GWALP23 is similar to that observed for GWALP23 itself (Figure 1). (Additional ²H NMR spectra for labeled alanines in the helical core of A^{4,5}GWALP23 are presented in Figure S5 of the Supporting Information.)

The F^{4,5}GWALP23 helix also shows low dynamic averaging, with an apparent tilt of ~21° in DLPC bilayer membranes [5]. An absence of side-chain hydrogen bonding may explain why the well behaved F^{4,5} peptide stands in contrast to the norm for other highly dynamic helices, such as the Y^{4,5}GWALP23 helix [18], that have multiple flanking aromatic groups capable of hydrogen bonding with the lipid head groups. Interestingly, substitution of non-

aromatic alanines 4 and 5 also leads to similar low dynamic averaging as observed for GWALP23 and F^{4,5}GWALP23. Indeed, A^{4,5}GWALP23 aligns well in bilayer membranes and displays distinct quadrupolar splittings for each side chain among the core Ala residues (Table 1). A semi-static tilt analysis, using a principal order parameter [23], reveals similar orientations for A^{4,5}GWALP23, F^{4,5}GWALP23, and GWALP23 helices in DLPC, DMPC and DOPC bilayer membranes (Figure 2; Table S1 of the Supporting Information). The apparent tilt of A^{4,5}GWALP23 ranges from ~8° in DOPC to ~18° from the bilayer normal of DLPC membranes (Table S1). An alternative modified Gaussian analysis of the dynamics confirms essentially the same peptide orientations in each of the lipid bilayer membranes (Table S2 of the Supporting Information).

Even as A^{4,5}GWALP23 has only one aromatic residue capable of hydrogen bonding (W19), there is not any obvious stabilizing residue, to limit the helix dynamics, near the N-terminus. The aromatic phenyl side chains of F^{4,5}GWALP23 show much less affinity than Trp for the interface and now have been replaced with methyl groups. The unprecedented low dynamic averaging exhibited by not only F^{4,5}- but more remarkably A^{4,5}GWALP23 tends to suggest that alternate factors are responsible for the stability of a particularly favored and well-defined average orientation of the transmembrane helix.

To test an alternative hypothesis, alanines 3 and 21 were chosen to receive ²H labels in both A^{4,5}GWALP23 and F^{4,5}GWALP23. Being 18 residues apart, alanines 3 and 21 are separated by exactly five helical turns [24] and hence would be geometrically over each other in identical radial positions in a perfect α -helix, meaning that their CD₃ quadrupolar splittings would superimpose regardless of the helix tilt. The actual ²H NMR results, however, show distinctly different $|v_q|$ values for alanines 3 and 21 in both F^{4,5}- and A^{4,5}GWALP23 (Figure 1). These results signify helix unwinding at both ends a heretofore not entirely expected or appreciated feature for “simple” hydrophobic transmembrane helices. Indeed, when the $|v_q|$ values for alanines 3 and 21 are compared to the quadrupolar wave plots for the core helix of either A^{4,5}GWALP23 or F^{4,5}GWALP23 (Figure 2), it is evident that residues 3 and 21 deviate not subtly but markedly from the helix geometry in all of the lipid-bilayer membranes. Similar partial helix unwinding near alanines 3 and 21 has been observed previously, for GWALP23 in DMPC [17], although the implications were not altogether recognized. Now we affirm similar “fraying” of GWALP23 in DOPC membranes (Table 1). Additionally, significant mobility has been observed by means of ¹³C NMR for the terminal residue A16 in WALP16 [25].

The terminal unwinding or end “fraying” involving at least three and perhaps four residues at each end of the GWALP family peptides can help to explain the high stability and low dynamics of a rather well-defined single transmembrane orientation for a neutral helix such as that of A^{4,5}GWALP23. Other than the indole ring of W19, there are no aromatic groups and no candidate side chains for favoring or stabilizing a particular orientation of the (tilted) transmembrane helix of A^{4,5}GWALP23. Importantly, there exist no side chains with hydrogen-bonding potential near the N-terminal of either A^{4,5}GWALP23 or F^{4,5}GWALP23. Attention is therefore directed toward the peptide backbone.

What feature is responsible for the “fraying” that exposes peptide backbone groups near the helix terminals of particular transmembrane peptides? Is the “fraying” a merely passive phenomenon? We think not. We reason that the partial unwinding is not passive but rather a critical stabilizing factor to define the geometry of the transmembrane helix orientation and limit the dynamics.

It is possible and indeed probable that helix unwinding outside of the core region could contribute to defining a particular orientation by allowing an uncoiled segment of peptide backbone to act as a “stake,” giving a large area of contact for hydrogen bonding at the membrane-water interface. It is known, for example, that uncompensated peptide backbone groups, because of very favorable atomic solvation energies [14], are driven to the membrane interfacial region [15] where possibilities for hydrogen bonding with lipids and water abound. Indeed a stabilizing hydrogen-bond network could easily be established among the peptide backbone groups from the unwound helix, water molecules and the polar lipid head groups.

We suggest therefore that the unwinding of the terminal segments (residues 1–3 or 1–4) is a vital feature for delineating a preferred (tilted) transmembrane orientation and minimizing the global dynamics, specifically limiting the rotational “slippage” about the helix axis [3–4], of bilayer-incorporated A^{4,5}GWALP23 and related peptide helices. With the core helix extending from about residue 4 ± 1 to about residue 20 ± 1 , the core helix length (at 1.5 Å per residue) is about 24 ± 3 Å. When tilted, the projected core dimension along the bilayer normal therefore is about 22.8 ± 2.8 Å when tilted 17°–18° in DLPC, and about 23.8 ± 3.0 Å when tilted 8° in DOPC (see Tables S1 and S2 of the Supporting Information). These projections should be compared to hydrophobic thicknesses (based on the Gibbs dividing surfaces) of 20.9 Å for DLPC [27] and 27.2 Å for DOPC [28]. Clearly, the extent of helix fraying can be different in DLPC and DOPC, yet the present ²H NMR results are not able to address such differences.

We observe, moreover, that the helix “fraying” could present challenges for particular molecular simulation methods, such as for instance some of the coarse-grained methods that may need to presume a particular unvarying peptide secondary structure. All-atom simulations, nevertheless, have shown fraying of proline-containing tails attached to transmembrane poly-Leu helices [29] and variable helicity of the C-terminal RKWQARQRGLQRF segment of the transmembrane PMP1 helix in lipid bilayers of POPC alone or with POPS [30]. Moreover, the favorable energetics of exposed interfacial peptide bonds could impart added significance to the loops and segments of extended backbone structure that connect the helices of multi-span transmembrane proteins. As opposed to being passive connectors, the loops and other inter-helix segments could assume added importance for establishing the conformations and regulating the functions of prominent classes of membrane proteins such as, for example, the seven-helix G-protein-coupled receptors [31–32]. It has been observed, for example, that helix fraying is a mechanism by which multi-span proteins such as G-protein-coupled receptors may adjust to a decrease in the hydrophobic thickness of the surrounding membrane [33–34].

We note finally that it would have been difficult to identify the intrinsic nature or significance of the transmembrane helix fraying in the absence of investigations that utilized designated model peptide-lipid systems.

Experimental Section

Commercial L-alanine-d₄ from Cambridge Isotope Laboratories (Andover, MA) was modified with an Fmoc group and was recrystallized from ethyl acetate:hexane, 80:20. Other protected amino acids, “Rink” amide resin and Wang resin were purchased from NovaBiochem (San Diego, CA). Peptides were synthesized on 0.1 mmol scale and purified as described previously [5, 18]. Typically, two deuterated alanines of differing isotope abundances were incorporated into each synthesized peptide.

Mechanically aligned samples for solid-state NMR spectroscopy (1/60, peptide/lipid, mol/mol) were prepared using DOPC, DMPC, or DLPC lipids from Avanti Polar Lipids (Alabaster, AL) and deuterium-depleted water (Cambridge; 45% w/w hydration), as described previously [17–18]. Bilayer alignment within each sample was confirmed using ³¹P NMR at 50 °C on a Bruker Avance 300 spectrometer (Billerica, MA) at both $\beta = 0^\circ$ (bilayer normal parallel to magnetic field) and $\beta = 90^\circ$ macroscopic sample orientations (Figure S3 of the Supporting Information). Deuterium NMR spectra were recorded at 50 °C using both sample orientations and a quadrupolar echo pulse sequence with 90 ms recycle delay, 3.2 μ s pulse length, and 115 μ s echo delay. An exponential weighting function with 100 Hz line broadening was applied prior to Fourier transformation.

Helix orientations were analyzed by means of the semi-static “GALA” method [23] and a modified Gaussian method using a fixed $\sigma\tau$, as described [5].

Supplementary Material

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Acknowledgments

We thank Ashley Martfeld and Jordana Thibado for helpful discussions. We thank two reviewers for uniformly constructive suggestions. This work was supported in part by U. S. National Science Foundation grant MCB 1327611, and by the Arkansas Biosciences Institute. The peptide, NMR and mass spectrometry facilities were supported in part by U. S. National Institutes of Health grants GM103429 and GM103450.

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Figure 1. ^2H NMR spectra for representative labeled alanines in $\text{A}^{4,5}\text{GWALP23}$ and GWALP23 , in oriented bilayers of DLPC. The numbers indicate the identities of the ^2H -Ala residues that yield the pairs of resonances: A. Core alanines 7 and 17 in $\text{A}^{4,5}\text{GWALP23}$. B. Core alanines 7 and 17 in GWALP23 . C. Juxta-terminal alanines 3 and 21 in $\text{F}^{4,5}\text{GWALP23}$. D. Juxta-terminal alanines 3 and 21 in $\text{A}^{4,5}\text{GWALP23}$. $\beta = 0^\circ$ sample orientation; 50°C .

Figure 2.

Quadrupolar wave plots for $A^{4,5}$ GWALP23 (red circles), $F^{4,5}$ GWALP23 (blue triangles), and the reference GWALP23 peptide (dashed curves; data points not shown but listed in Table 1) in (A) DOPC, and (B) DLPC. The $|\nu_q|$ values for alanines 3 and 21 (numbered, at helical wheel position 200°) are clearly off of the helical wave defined by the six core alanine residues. C. Models for $A^{4,5}$ GWALP23 (left) and $F^{4,5}$ GWALP23 (right), showing the observed tilted core helix orientations in DLPC, as well as schematic representations of the unwinding of the helix terminals for the bilayer-incorporated peptides. The locations of

W19 and residues 3–5 are illustrated on a ribbon helix, drawn using PyMOL ^[26]. The depicted side chain orientations are arbitrary.

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Table 1

^2H NMR quadrupolar splitting magnitudes ($|\nu_q|$, in kHz) for labeled core alanine and flanking alanine (3 and 21) CD_3 groups in $\text{A}^{4,5}\text{GWALP23}$, $\text{F}^{4,5}\text{GWALP23}$ and GWALP23 (having L^4W^5)^[a]

Ala-d ₄	A ^{4,5}	F ^{4,5} [b] x	L ⁴ W ⁵ [b]	Lipid
7	23.2	23.7	26.4	DLPC
9	19.0	23.5	25.5	DLPC
11	23.6	25.7	26.9	DLPC
13	11.7	19.6	14.6	DLPC
15	18.7	23.4	20.7	DLPC
17	6.6	1.8	3.4	DLPC
3	22.3	20.6	--	DLPC
21	10.2	2.6	--	DLPC
7	20.4	20.3	21.9	DMPC
9	10.8	--	8.9	DMPC
11	20.6	20.6	20.9	DMPC
13	9.0	--	3.8	DMPC
15	18.8	20.6	17.6	DMPC
17	--	3.8	2.9	DMPC
3	19.6	20.4	10.8	DMPC
21	6.3	2.0	3.2	DMPC
7	15.4	16.2	16.6	DOPC
9	4.0	0.8	1.7	DOPC
11	18.6	18.6	16.7	DOPC
13	5.2	0.8	1.5	DOPC
15	16.4	18.6	15.4	DOPC
17	0.5	1.9	2.6	DOPC
3	14.3	11.8	10.4	DOPC
21	1.8	2.2	2.7	DOPC

^[a] Sample orientation is $\beta=0^\circ$, in which the bilayer normal is parallel to the external magnetic field. Each value (in kHz) is the average of the magnitude observed at $\beta=0^\circ$ and twice the magnitude observed for a $\beta=90^\circ$ sample orientation. Values that are absent (--) were not recorded. The labeled alanines (Ala-d₄) are identified, and the positions of the variable amino acid residues are indicated as A^{4,5}, F^{4,5}, or L⁴W⁵ for GWALP23 itself.

^[b] Some of the data points for core alanines in $\text{F}^{4,5}\text{GWALP23}$ and GWALP23 were presented in references [17] and [5].